AUTOANTIBODIES AND THE AUTOIMMUNE DISEASES*

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THE concept of autoimmunity was considered early in the development of immunology. Erhlich has often been quoted on his views of "horror autotoxicus."[1] During the last decade, theoretical and experimental considerations have strongly indicated that production of autoantibodies is not only biologically permissible, but is an integral component of the regulation of the immune system.[2] The autoantibodies so produced are directed against the combining site of other specific antibodies that have unique configurations called idiotypes. The antiantibodies are thus called anti-idiotypic antibodies.

The case for autoantibodies now appears strong. For many years clinicians have noted the association of serological activities with various disease states. These serological activities appear to be antibody-antigen reactions. Arguments that these were not true autoantibodies were based largely on the *à priori* assumption that the production of such antibodies was either impossible or highly abnormal. Those who held that autoantibody production was not biologically permissible argued that the serological activities being measured involved denatured protein or nucleic acids as antigens. Those holding it to be distinctly pathological developed theories such as that of the "forbidden clone." It may well be that certain classes of autoantibodies are common and perhaps necessary, these being anti-idiotypes, while others such as antinuclear antibodies may represent the emergence of such forbidden clones.

As we have learned more about the regulation of the immune system, another mechanism has been described to explain the emergence of autoantibodies. The phenomenon of tolerance, wherein an animal accepts a homograft or receives doses of proven antigen without an immune

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response was originally investigated for its utility in solving problems of tissue grafting. Subsequent studies have indicated that tolerance is a general biologic phenomenon, having several mechanisms, and is the usual state existing between an organism's immune system and some or all of the constituent proteins of the same organism. Thus, thyroglobulin is not thought to be sequestered from the human immune system, but to exist in a state of tolerance such that the normal immune mechanisms do not, or cannot respond.[3] Another mechanism for autoimmunity can now be suggested. Just as tolerance to given antigens can be developed in experimental animals, techniques have been used which cause experimental animals to produce antibodies to their own protein, thyroglobulin being a notable example. As these techniques involve slight alterations in protein configuration or in the amount of protein released from the gland, and as other events could change the quantity of protein released from tissue, it is conceivable that natural phenomena could lead to disruption of natural tolerance and production of autoantibodies.

While the arguments for and against autosensitization have held sway in turn, clinicians have utilized the observed serological reactions by correlating the presence and quantity of various posited autoantibodies with the presence and activity of a variety of diseases. The validity of these observations are independent of the theoretical models used to explain them. The determinations of an "antiantibody," the rheumatoid factor, are derived from Cecil's original studies demonstrating that the sera of patients with rheumatoid arthritis agglutinate streptococci under certain well defined conditions.[4] Although these studies were started as a search for antibodies to the streptococci, it was soon apparent that the test measured the ability of rheumatoid sera to agglutinate streptococci already coated with antibody, i.e., the presence of an antibodylike material directed against human gamma globulin. The test most commonly used for the detection of rheumatoid factor is latex agglutination.[5] In this determination, an IgM immunoglobulin in the patient's serum reacts in an antibodylike fashion with partially denatured IgG absorbed to the surface of the latex particles. This is only one of several techniques used to detect rheumatoid factor, each of which utilizes the adsorption or immune reaction of IgG with a solid phase, and the agglutination of that solid phase by a factor in the patient's serum. In the sheep cell agglutination test, the IgG is not of human origin, but is rabbit antibody to the sheep cells. Some 80% of rheumatoid patients have a positive rheumatoid factor,

and in general these patients have more severe disease. Patients with nodular rheumatoid arthritis have positive serologic tests in a high percentage of cases, but even in this group it is not universal.

Rheumatoid factor can be detected in the serum of patients with subacute bacterial endocarditis and other chronic infections,[6] as well as in the serum of patients with systemic lupus erythematosus and, indeed, in individuals without disease, particularly in the aged population.[7] The titers in nonrheumatoid arthritis patients is usually low, and, as in the case of subacute bacterial endocarditis, may disappear when the disease is treated. Thus, a positive rheumatoid factor may be confirmatory in given clinical situations, but is not diagnostic of rheumatoid arthritis, nor does the absence of the factor exclude that disease. In practical terms, a clinician seeing a patient with advanced erosive arthritis may utilize this laboratory test to confirm the diagnosis of rheumatoid arthritis. A positive test, especially in high titer, would ordinarily terminate the diagnostic process. A negative test would indicate that further investigations into the nature of the arthritis were needed. Diseases such as ankylosing spondylitis with peripheral joint involvement, certain types of juvenile rheumatoid arthritis, psoriatic arthritis, and even tophaceous gout can mimic rheumatoid arthritis but are ordinarily seronegative.

The standard clinical tests do not detect non-IgM rheumatoid factor, that is, IgG or IgA rheumatoid factor. While it had been supposed that seronegative rheumatoid patients are frequently positive for non-IgM rheumatoid factor, this has not been proven universally to be the case.[8]

Like the rheumatoid factor for rheumatoid arthritis, the serological tests for systemic lupus erythematosus (SLE) are confirmatory but not in every case diagnostic. While it is distinctly unusual for patients with systemic lupus erythematosus to be seronegative, patients with diseases other than lupus may demonstrate positive antinuclear factors.[9] The LE cell test, historically the oldest of the serological tests for systemic lupus erythematosus, involves the phagocytosis of opsonized nuclear material. Opsonization is accomplished by a specific component of the patient's serum, anti-DNA-nucleoprotein, and is possibly aided by complement components. It is one of the more specific tests but it is not as sensitive as the fluorescent antinuclear antibody determination. In the latter test, patient's serum is layered over cryostat cut tissue sections. Antibody to nuclear components reacts with their antigens and remain bound to the sections following washing. The antibody can then be detected using fluorescence labelled

rabbit or goat antihuman gamma globulin. The detection of human gamma globulin outlining the entire nucleus or a portion of the nucleus indicates a positive antinuclear antibody test. Patients with systemic lupus erythematosus almost universally show positive antinuclear antibody tests. The pattern of the test may give a clue as to the activity of the disease, the rim or shaggy pattern being associated with a high anti-DNA titer, usually a hallmark of active disease. The presence of anti-DNA in the patient's serum can be detected by the demonstration that radioisotope labelled native DNA will coprecipitate with the patient's gamma globulin.[10]

An extraordinary variety of nuclear factors have been shown to react with the serum of patients with systemic lupus erythematosus or other rheumatologic diseases. Notable among these are the extractable nuclear antigens, that is, antigens readily soluble in saline and thus easily removed from nuclear preparations. Serum from patients with lupus will often show reactivity with one extractable nuclear antigen, the Sm antigen which is ribonuclease insensitive. Patients with mixed connective tissue disease will have high titer serological reactivity with ribonuclease sensitive extractable nuclear antigen.[11] Other nuclear antigens, the SS-B or Ha antigen, are associated with primary Sjogren's syndrome or Sjogren's syndrome associated with systemic lupus erythematosus, while another serologic abnormality, the rheumatoid arthritis-associated protein (RAP), is found in the serum of patients with Sjogren's syndrome associated with rheumatoid arthritis.[12] This latter antibody reacts specifically with the rheumatoid arthritis-associated nuclear antigen found only in the nuclei of B lymphocytes which have been transformed by the Ebstein-Barr virus.

This spectrum of different antibody-antigen systems, collectively called the antinuclear antibodies, are at many levels diagnostically useful. High extractable nuclear antigen titers in a patient with sclerodermatous and myositic components indicate mixed connective tissue disease, and further indicate a relatively benign prognosis, while high anti-DNA antibody titers in a patient with systemic lupus erythematosus can portend severe renal involvement.

It must be recognized that the etiology of these diseases is a matter of conjecture, but, unlike the presence of rheumatoid factor, where no firm pathogenetic role has been established for this serum factor, antinuclear antibodies, whatever their cause, may lead to functional and anatomical changes. Thus, anti-DNA-DNA complexes may well deposit in the glomerulus and lead to the renal involvement of lupus erythematosus.[13]

Direct action of certain antibodies may be a major factor in the pathogenesis of disease. This type of reaction has been classified as a Type II Coomb's and Gell reaction.[14] The list of target antigens involved in such reactions is indeed long. Probably because of the ease of detection of both the antibody and the antigen as well as because the effects of the antibody-antigen reaction are rapidly evident, antibodies to such formed blood elements as erythrocytes, platelets, and leukocytes as well as lymphocytes have been described. These autoantibodies may be seen following viral or other infectious disease such as the cold agglutinins following infections with mycoplasma, or may appear in association with systemic lupus erythematosus or a related disease. Thrombocytopenia and autoimmune hemolytic anemias are not uncommon in lupus, and it is suspected that antibodies to other blood elements are frequently present, if not often recognized. The stimulus for the production of such autoantibodies may be a "breaking of tolerance," as might be the case when erythrocyte or platelet membranes are associated with viral protein. In lupus it is suggested that derangements of the immune system, perhaps resulting from a lack of T suppressor cells, may be the initiating event in the formation of these autoantibodies. Isolated instances of autoimmune hemolytic anemia, or thrombocytopenic purpura, unassociated with other systemic diseases, are not uncommon. The diagnosis of these autoimmune diseases rests on recognition of the clinical process, i.e., hemolysis or bleeding associated with thrombocytopenia. Under ordinary circumstances, a patient's serum contains antibodies which react with red cells or platelets in *in vitro* studies. Antierythrocyte antibodies can usually be detected on the surface of the patient's own red cells by reacting the patient's washed red cells with an antiglobulin preparation. Agglutination will occur only if the cells are sensitized, that is, coated with antibody. A unique example of autoantibodies causing severe morbidity and, indeed, frequently death, is found in Goodpasture's syndrome. Here a patient develops antibodies to basement membrane antigens, common to both the lung and the kidney. Severe hemoptysis and rapid development of renal failure ensue. Positive diagnosis in this situation is ordinarily made by detecting basement membrane bound immunoglobulin using the immunofluorescent technique.

Other systems of autoantibodies are now being described in which the antibody is directed against a receptor located on the surface of a given cell. In Grave's disease, antibodies to thyrotrophic stimulating hormone

receptors stimulate the thyroid, bypassing the usual feedback mechanisms involved in thyroid-stimulating hormone.[15] This results in hyperthyroidism. The ophthalmologic changes frequently seen in this disease may be secondary to immune complexes thus formed which may lodge in the orbit. Myasthenia gravis involves an antibody to the acetylcholine receptor which blocks the action of acetylcholine and inhibits neuromuscular transmission. In certain rare forms of insulin resistant diabetes associated with acanthosis nigricans, an antibody to the insulin receptor blocks the action of that hormone.

Autoantibodies have been detected in many diseases but, as in rheumatoid arthritis, no pathogenetic mechanism for the antibody has as yet been determined and these serological activities may result from, rather than, cause the disease. Such diseases are Hashimoto's thyroiditis in which antithyroglobulin antibodies are seen in 50% to 60% of cases, and pernicious anemia in which antiparietal cell, antibody, and antiintrinsic factor antibodies are seen. Celiac disease and dermatitis herpetiformis are associated with antireticulin antibody, biliary cirrhosis with antimitochondrial antibodies, and chronic active hepatitis with antismooth muscle antibody.

Finally, antibodies to various skin elements such as those seen in pemphigus vulgaris should be mentioned.

Numerous other autoantibodies have been described and may either be of value in the diagnosis or in determining the pathogenesis of various diseases. In each case the physician must make a clinical judgment as to the importance of the antibody, both in the pathogenetic process and in the diagnostic process. Only by carefully reviewing the medical experience and by correlating laboratory findings with historical and physical data can proper diagnoses and treatment be approached.

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